Gibberellin-Induced Changes in the Populations of Translatable mRNAs and Accumulated Polypeptides in Dwarfs of Maize and Pea[1]

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ABSTRACT

Two-dimensional gel electrophoresis was used to characterize the molecular mechanism of gibberellin-induced stem elongation in maize and pea. Dwarf mutants of maize (d-5) and pea (Progress No. 9) lack endogenous gibberellin (GA₁) but become phenotypically normal with exogenous applications of this hormone. Sections from either etiolated maize or green pea seedlings were incubated in the presence of [³⁵S] methionine for 3 hours with or without gibberellin. Labeled proteins from soluble and particulate fractions were analyzed by two-dimensional gel electrophoresis and specific changes in the patterns of protein synthesis were observed upon treatment with gibberellin. Polyadenylated mRNAs from etiolated or green maize shoots and green pea epicotyls treated or not with gibberellin (a 0.5 to 16 hour time course) were assayed by translation in a rabbit reticulocyte extract and separation of products by two-dimensional gel electrophoresis. Both increases and decreases in the levels of specific polypeptides were seen for pea and corn, and these changes were observed within 30 minutes of treatment with gibberellin. Together, these data indicate that gibberellin induces changes in the expression of a subset of gene products within elongating dwarfs. This may be due to changes in transcription rate, mRNA stability, or increased efficiency of translation of certain mRNAs.

A role for gibberellins has been implicated in a wide range of developmental processes in plants, from seed development through senescence (13). In only one tissue, however, the aleurone of germinating wheat and barley, has any progress been made towards understanding GA²-regulated development in molecular terms (1, 5). One well-described physiological phenomenon for which a role for GA has been implicated is stem elongation. Genetic and biochemical studies performed on dwarf mutant plants have identified a class to dwarfs which respond to exogenously added GA (13, 18, 19). These plants are generally only about 20% the height of normal plants in the absence of GA, but grow to full height following GA application. The biochemical bases for some of the mutations which lead to the dwarf phenotype have been defined (20, 23). In maize, for example, the d-5 mutant is blocked between copalylpyrophosphate and ent-kaurene in the early-13-hydroxylation pathway leading to biologically active GA₁ (9). The dwarf-5 mutant,

therefore, has little or no endogenous GA. GA-biosynthetic mutants of pea have also been described (20, 21). Progress No. 9 (lele) is generally thought to be blocked in the conversion between GA₂₀ and GA₁ when grown in the light (20); however, recent data indicate that this mutation is leaky (10). Progress No. 9 has endogenous GAs, but since the biologically active form, GA₁, is present in only trace amounts in light grown plants, this plant exhibits the dwarf phenotype.

Although the gibberellins have long been implicated in the regulation of stem growth in plants, their precise role is still unclear. The result of GA application to dwarf plants is enhanced cell division and cell elongation. Basic molecular questions relevant to GA-induced growth, however, remain unanswered. There is little or no information, for instance, on the types of proteins required for GA-induced stem elongation or on the roles these proteins might play in the process of cell division and cell elongation. There is some evidence that GA enhances protein synthesis nonspecifically during elongation (26), but whether GA alters preexisting proteins or enhances or represses the synthesis of a limited number of polypeptides is unknown.

To answer some of these questions, and to determine the feasibility of cloning genes whose expression is dependent on the presence of GA, we have utilized dwarf varieties of corn (d-5) and pea (Progress No. 9) which lack the biologically active
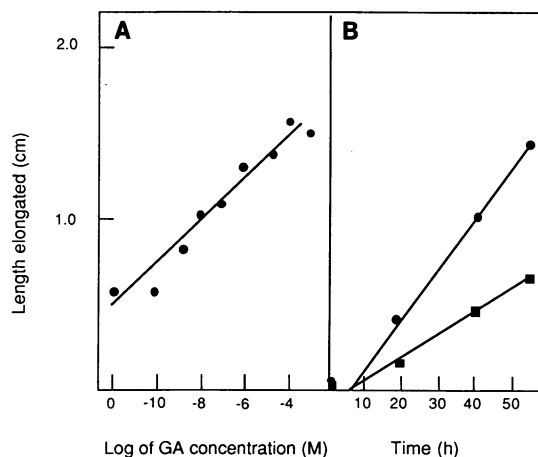

FIG. 1. A, Elongation of etiolated maize shoot sections after incubation for 48 h in the dark in the presence of various concentrations of GA₃. The original length of each section was 1.4 cm. Each point is the average of 10 independent measurements. B, Elongation of etiolated maize shoot sections as a function of time over a 60 h incubation period. Each point is the average of 30 independent measurements. (●), Elongation in the presence of GA; (■), elongation in the absence of GA.

[2] Abbreviations: GA, gibberellin; IEF, isoelectric focusing; CHAPS, (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate).
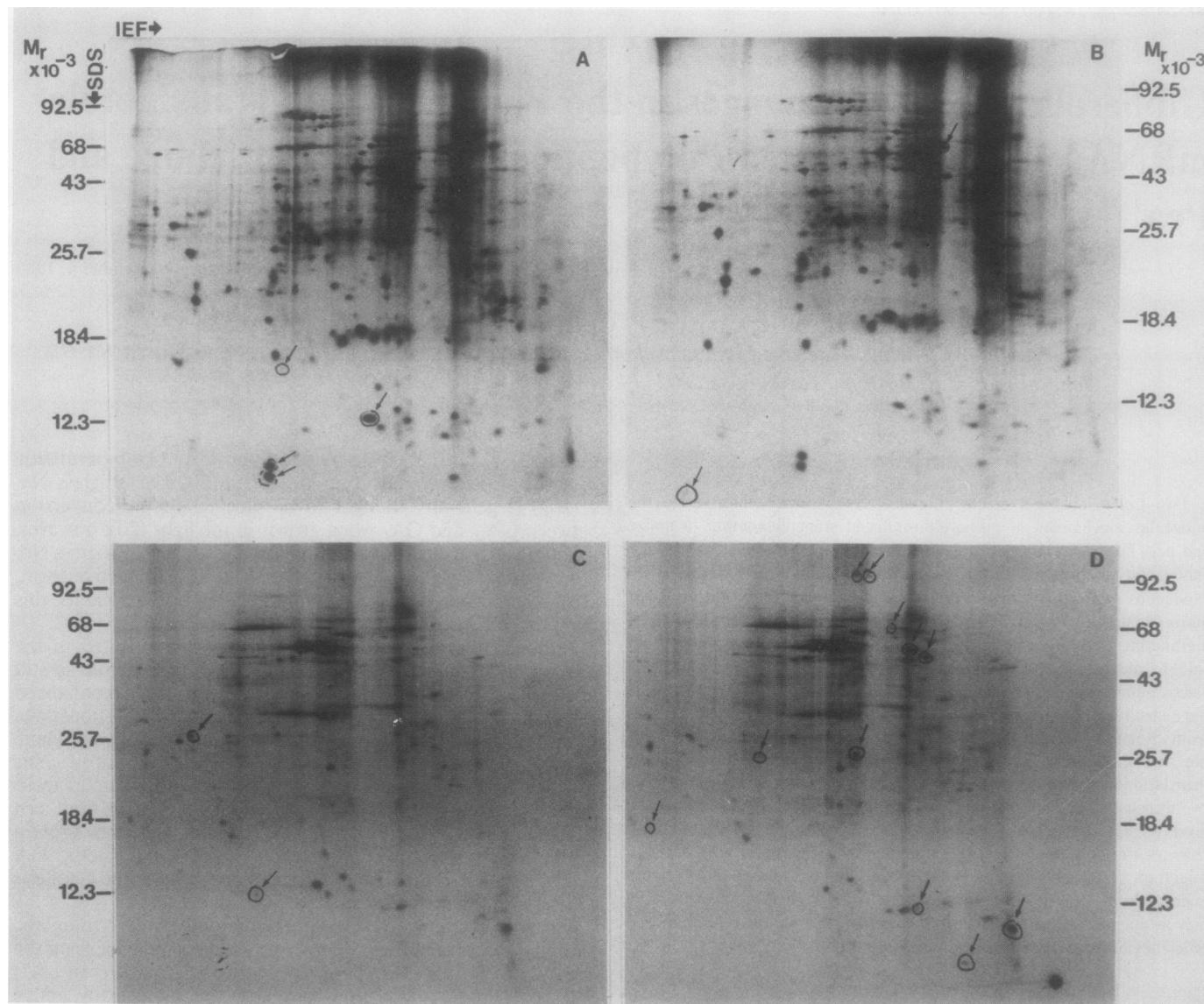
FIG. 2. Fluorographs of [^{35}S]methionine-labeled polypeptides from maize shoots resolved by electrophoresis on two-dimensional gels. The samples are: A, the soluble portion from untreated segments; B, the soluble fraction from GA-treated segments; C, the particulate fraction from untreated segments; and D, the particulate fraction from GA-treated segments. The arrows in panels (A) and (C) point to polypeptides whose synthesis is repressed by treatment with GA. In panels (B) and (D), the arrows point to polypeptides whose synthesis is increased or induced by the presence of GA.

gibberellin, GA$_1$. We chose these two particular dwarfs because of the numerous physiological and biochemical studies which have been previously performed with them. Both pea and corn were examined to compare the GA response in monocots and dicots, and because we wanted to assess which system would be most useful for molecular analyses. We have utilized *in vivo* protein labeling with [^{35}S]methionine coupled with two-dimensional gel electrophoresis to obtain a general picture of GA-induced polypeptide changes. In addition, cell-free translation of purified mRNA followed by two-dimensional gel electrophoresis was used to assess the spectrum of changes in the mRNA pool at discrete times after GA application.

MATERIALS AND METHODS

Plant Material. Maize seeds (*Zea mays* var dwarf *d-5*) and pea seeds (*Pisum sativum* var Progress No. 9) were planted in vermiculite and germinated in the light. After 7 d, the dwarf corn was sprayed with either 10 μM GA$_3$ or water. (GA$_3$ was used because it is commercially available and known to be biologically active in these two dwarfs.) Plants were harvested after the various times indicated in the text and immediately frozen in liquid N$_2$ until sample preparation. For peas, the seedlings were sprayed at 11 d with either GA$_3$ or water. At the times described in "Results," the shoots were harvested, leaves removed, and the stems frozen in liquid N$_2$.

Some of the *dwarf-5* experiments were performed on segments from etiolated plants. Inasmuch as it has been reported that dwarf corn responds to GA in the dark (22), and since growing corn in the dark greatly improved our ability to isolate RNA and to resolve polypeptides on two-dimensional gels, most of the experiments done with the maize dwarf, *d-5*, were performed with etiolated 5 d seedlings. The seeds were planted in coarse vermiculite and germinated in the dark at 28°C. After 5 d, the shoots were excised, and floated in a solution containing sucrose, 2%; chloramphenicol, 50 μg/ml; and 10 mM K-phosphate (pH 6), with or without GA$_3$. The incubations were performed in the
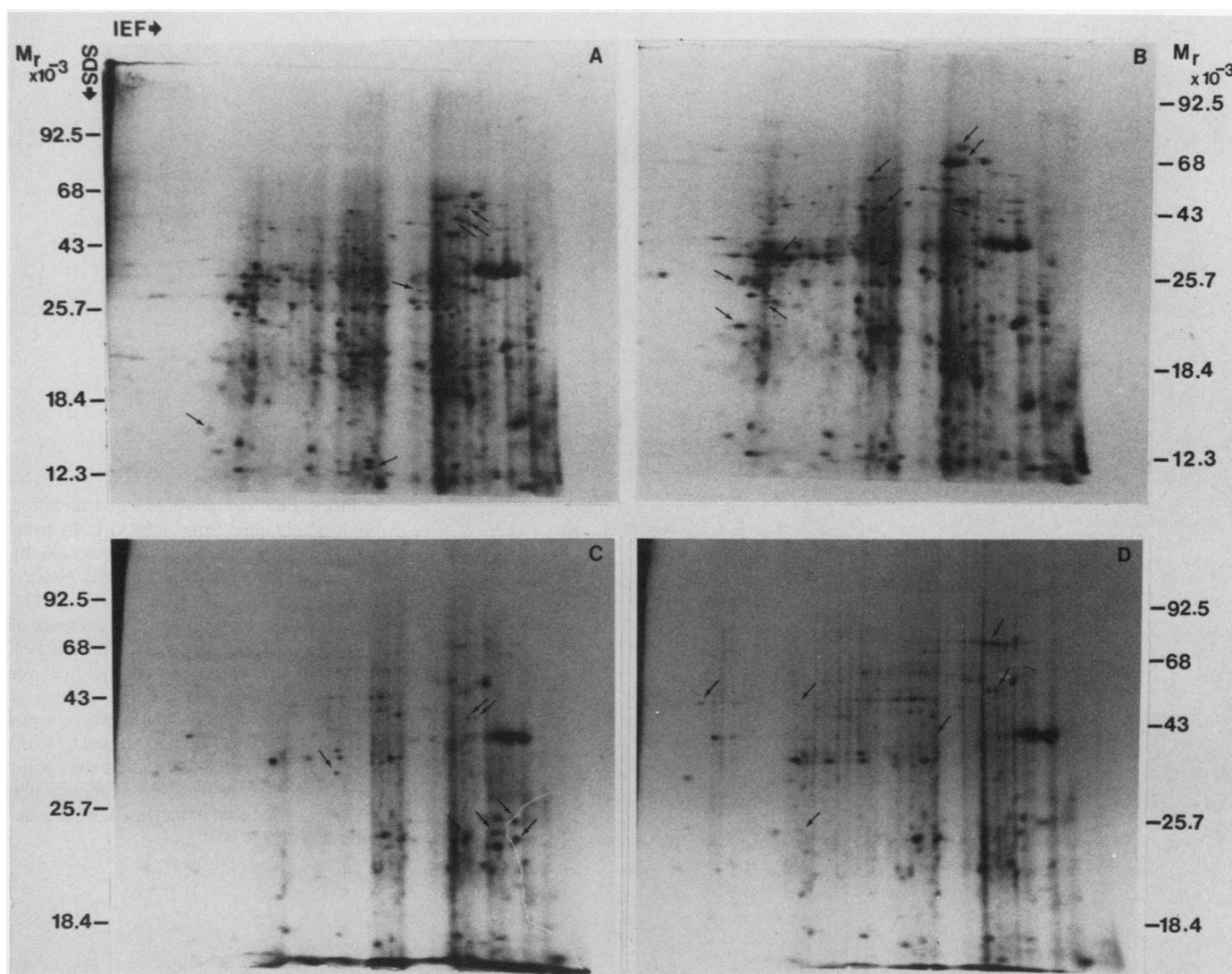
FIG. 3. Fluorographs of [^{35}S]methionine-labeled polypeptides from pea stem sections resolved by electrophoresis on two-dimensional gels. The samples are as described in the legend to Figure 2.

dark in a shaking water bath set at 28°C. The optimal concentration of GA$_3$ was determined by the experiment depicted in Figure 1. After incubating for the various times described, the shoots were rinsed in H$_2$O, quickly frozen in liquid N$_2$, and stored at −70°C.

Radioactive Labeling of Proteins in Shoot and Stem Sections. The methods used for labeling corn and pea were essentially identical and were adapted from Zurfluh and Guilfoyle (28). For *in vivo* labeling, excised corn shoots (etiolated) or pea stems were treated for 3 h in a solution which was 2% sucrose; 10 mM sodium phosphate (pH 6.0); chloramphenicol (50 μg/ml); [^{35}S] methionine, 100 μCi/ml; with or without GA$_3$ at 10 μM. Corn was treated in the dark. After incubation, the segments were rinsed in H$_2$O and frozen immediately in liquid N$_2$ prior to processing.

Preparation of *in Vivo* Labeled Proteins for Polyacrylamide Gel Electrophoresis. The samples were processed as follows: Briefly, frozen tissue was ground with a mortar and pestel. The sample was then homogenized for 2 min in a Polytron in a buffer containing sucrose, 250 mM; Tris-HCl, 50 mM (pH 7.4); MgCl$_2$, 5 mM; and KCl, 50 mM. The homogenate was filtered through one layer of Miracloth and two layers of cheesecloth to remove the intact tissue. To eliminate streaking artifacts on two-dimen-

sional gels, the green tissue was extracted with insoluble PVP for binding phenols, and washed with sodium ascorbate (7). After homogenization, the tissue was fractionated as follows: Centrifugation at 10,000g for 10 min was used to remove large tissue fragments. The supernatant fraction was removed and further fractionated into soluble (100,000g supernatant fraction) and particulate (100,000g pellet) phases. The soluble fraction was precipitated with 5 volumes of cold acetone at −20°C to concentrate the proteins and then resuspended in lysis buffer for two-dimensional gel electrophoresis (16). The particulate portion (pellet) was directly resuspended in lysis buffer for electrophoresis. Those samples which were not analyzed immediatley were quickly frozen in liquid N$_2$. Protein concentrations were measured by the method of Bradford (3), using bovine γ-globulin as standard. The amount of [^{35}S]methionine incorporated into protein was measured after TCA-precipitating an aliquot of the samples onto Whatman 3MM paper, boiling for 5 min, rinsing with ethanol, then acetone, and drying.

Purification of Polyadenylated mRNA. Poly(A) RNA used for *in vitro* translation was isolated from corn and pea using standard procedures. Total nucleic acids were extracted from the frozen tissue by homogenization in a Polytron in the presence of SDS and phenol (8). RNA was purified by centrifugation through a
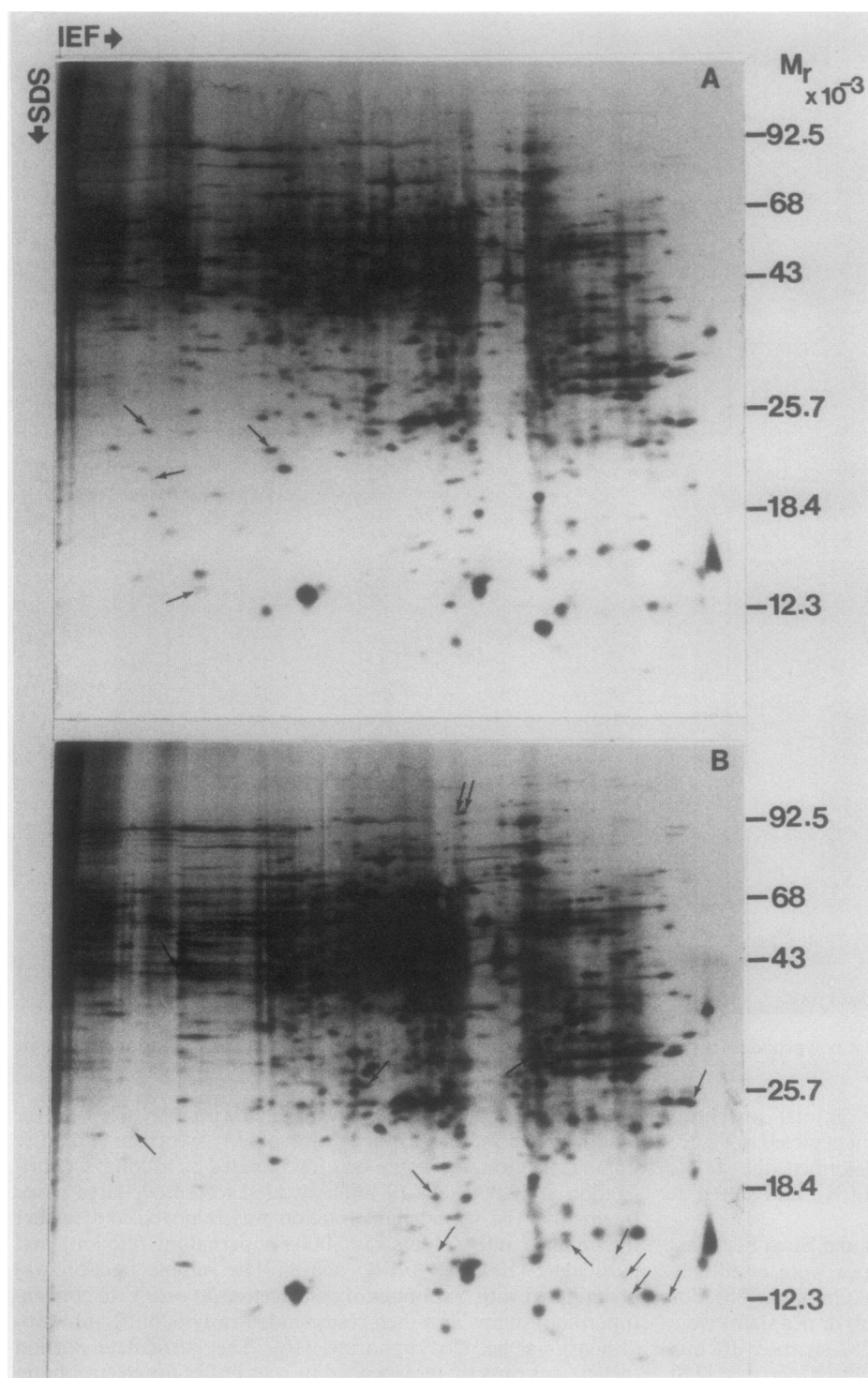
FIG. 4. Fluorographs of *in vitro* translation products obtained from poly(A) RNA from etiolated maize seedlings and resolved by two-dimensional gel electrophoresis. Excised shoots were incubated in the absence of GA for 0.5 h (A), or for 0.5 h in the presence of 10 μM GA$_3$ (B). *In vitro* translation was performed in a rabbit reticulocyte cell-free proteins synthesizing system. The arrows in panel (A) point to polypeptides whose levels of translatable mRNAs are repressed by treatment with GA. In panel (B), the arrows point to polypeptides whose levels of translatable mRNAs are induced or increased by the presence of GA.

5.7 M CsCl cushion. Poly(A) mRNA was isolated by affinity chromatography on oligo(dT)-cellulose as described (12), except that the elution buffer was maintained at 50°C.

Cell-Free Protein Synthesis and Gel Electrophoresis. Poly(A) RNA was translated in rabbit reticulocyte or wheat germ lysates (New England Nuclear) in the presence of [35S]methionine as described by the manufacturer. Cell-free protein synthesis was terminated by placing the reaction mixture on ice. An aliquot of the sample was TCA-precipiated (hot) as described above. Five volumes of acetone were added to the remainder of the sample and the proteins allowed to precipitate at −20°C for at least 1 h. The acetone precipitates were pelleted by centrifugation in a

Beckman microfuge for 5 min at 4°C, dried, and dissolved in 75 to 100 μl of O'Farrell's lysis buffer (16), except that the detergent used was CHAPS (17). The insoluble proteins were removed by another centrifugation in the microfuge. An equal number of counts for treated and untreated samples were loaded on first-dimension IEF gels to facilitate comparison between samples after electrophoresis.

Translation products were separated in two dimensions by IEF gel electrophoresis in the first dimension and a 12% (w/v) acrylamide SDS gel in the second dimension as described by O'Farrell (16), with the following modifications. The detergent used in the lysis buffer and in the IEF dimension was CHAPS

Table I. *Summary of Polypeptide Changes in Maize as a Result of GA$_3$ Treatment*[a]

Synthesis Repressed		Synthesis Enhanced		
			In vitro	
In vivo	In vitro[b]	In vivo	Etiolated tissue[b]	Green[c]
26.5[d]	23.5[d]	94.4[d]	94.3[d]	36.6
15.8	22.0	92.9[d]	93.3[d]	35.3
12.3[d]	20.3	68.0	42	28.3[e]
11.8	13.5[d]	57.2	31	26.1
9.7		57.1	28.5[e]	25.1
9.1		52	24.7[d]	10.1
		25.1[d]	23.9	
		22.6	17.7	
		18	14.5	
		11.5	14.3	
		9.3	14	
		8.7	13.3	
		7.3	12.2	
			11.3	

[a] Tabulated are molecular weights relative to known protein standards. [b] Shown are the values for the 0.5 h time point. The changes are the same for 2, 4, 8, and 16 h. [c] Shown are the values for the 2 h time point. The changes are the same for 4, 8, 24, and 48 h. [d] Refers to changes seen in polypeptides both during *in vivo* and *in vitro* labeling. [e] Refers to changes seen in polypeptides in both green and etiolated tissue.

(17). The anode solution was 25 mM H$_3$PO$_4$, instead of 10 mM; and the cathode solution was 50 mM NaOH, instead of 25 mM NaOH. IEF was conducted for 12 h at 800 V. After electrophoresis in the second dimension, slab gels were fixed in a solution of 10% acetic acid, 25% ethanol for at least 1 h. The gels were fluorographed using En^{3}Hance prior to drying onto Whatman 3MM paper. The dried gels were exposed to Kodak X-Omat AR x-ray film. A typical exposure time for *in vitro* translated proteins was about 12 to 16 h. The *in vivo* labeled samples were exposed to film for about 5 d.

Materials. Translation-grade [^{35}S]methionine (1200 Ci/ mmol), rabbit reticulocyte lysate, wheat germ lysate, and En^{3}Hance were from New England Nuclear. CHAPS and GA$_3$ were from Sigma Chemical Co. The oligo(dT)-cellulose was from either Collaborative Research or from Bethesda Research Laboratories. Dwarf corn (*d-5*) was a generous gift of Dr. B. O. Phinney (UCLA) or was from Carolina Biologicals. Progress No. 9 was obtained from Agway Seed Co.

RESULTS AND DISCUSSION

Response of Etiolated *d-5* Maize and Progress No. 9 Pea Sections to Exogenously Added GA$_3$. It has been reported that dwarf corn responds to GA in the dark (22). The use of etiolated maize seedlings greatly improved our ability to isolate RNA and to resolve polypeptides on two-dimensional gels; however, the precise conditions for incubating segments in the dark were not known. Figure 1A shows the amount of elongation of etiolated maize shoot sections after incubation for 48 h in the dark with various concentrations of GA$_3$. Based on these data, a concentration of 10 μM GA$_3$ was used for all subsequent experiments. Figure 1B depicts the elongation of etiolated maize shoot sections over a 60 h incubation period. After a short lag time, the rate of elongation was approximately constant, and those segments which were incubated in the presence of 10 μM GA, elongated at a faster rate.

When similar experiments were attempted with Progress No. 9, we found that etiolated peas did not respond to the addition

of GA. This was true for sprayed seedlings as well as for pea stem segments incubated in the presence of GA$_3$ or GA$_3$ plus auxin. Therefore, we performed all the pea experiments on 11-d-old green pea seedlings, sprayed with 10 μM GA$_3$. The elongation data for dwarf pea plants grown under these conditions have been published elsewhere (10).

Patterns of Protein Synthesis in Etiolated Maize Shoot Sections and Pea Stems. Because very little is known about the molecular nature of the growth response induced by GA in plants, we decided to look at the accumulation of newly synthesized polypeptides in elongating dwarfs. To do this, either pea stem or corn shoot segments were incubated for 3 h with or without GA$_3$ in the presence of [^{35}S]methionine to label cellular proteins. The cells were lysed and fractionated into crude soluble and particulate phases. The crude particulate fraction contained cell membrane and cell wall constituents and any membrane fragments from broken mitochondria and chloroplasts. Both fractions were solubilized and proteins separated by two-dimensional gel electrophoresis. Several differences were noticed in the polypeptide patterns of untreated and GA-treated corn and pea sections.

Figure 2 shows the differences observed in the polypeptide profiles of etiolated maize sections. Comparison of about 300 spots in the soluble fraction (panels A and B), reveals that the most significant differences between untreated and treated maize seedlings are the decrease of four low M_r polypeptides and the appearance of two polypeptides, with M_rs of 8 and 55 kD. By far, though, the most significant differences seen in the protein profiles of untreated and GA-treated maize sections occurred in the particulate phase of the cell (Fig. 2, panels C and D). Here, the appearance of 11 new polypeptides (of about 100 total examined) occurs in the presence of GA$_3$. Also, the disappearance of two polypeptides was seen (Fig. 2C). These results were verified over repeat trials.

The results for labeled pea segments were similar, but not identical (Fig. 3, A–D). Analysis of the soluble portion reveals the appearance of nine new polypeptides in the presence of GA$_3$ and the disappearance of seven. The particulate phase profiles of pea also showed changes, though not as striking as with maize. Of 100 total polypeptide spots examined, we observed the appearance of six new polypeptides. concomitant with the disappearance of six protein spots (Fig. 3, C and D). As with maize, a large percentage of the total spots analyzed in the particulate phase was changed. Although there appeared to be some proteolysis in these samples, when the experiment was repeated in the presence of protease inhibitors, no significant increase in high mol wt proteins were observed.

From the experiments depicted in Figures 2 and 3, we conclude that GA alters the pattern of synthesis in elongating maize shoot sections and pea stems, especially in the particulate fraction. The method by which these changes are effected is not known. GA could promote the synthesis of specific polypeptides, repress the synthesis of other polypeptides, or cause charge modification or other posttranslational modifications of proteins synthesized in the absence of GA.

Similar experiments have been conducted with other plant hormones in a variety of tissue types. Auxins applied to elongating and basal sections of soybean hypocotyl have been shown to alter the levels of a small percent (3%) of the total proteins analyzed (28). Similarly, in studies regarding the effects of cytokinin on protein accumulation in pumpkin cotyledons, about 50% of the total proteins examined showed differences in the presence of cytokinin (6). The plant growth regulator ethylene has also been shown to alter gene expression, particularly the activities of phenylalanine ammonia lyase (4) and chitinase (2). These changes are seen within 24 h of treatment with ethylene. Finally, one can compare the effects of GA on stem elongation
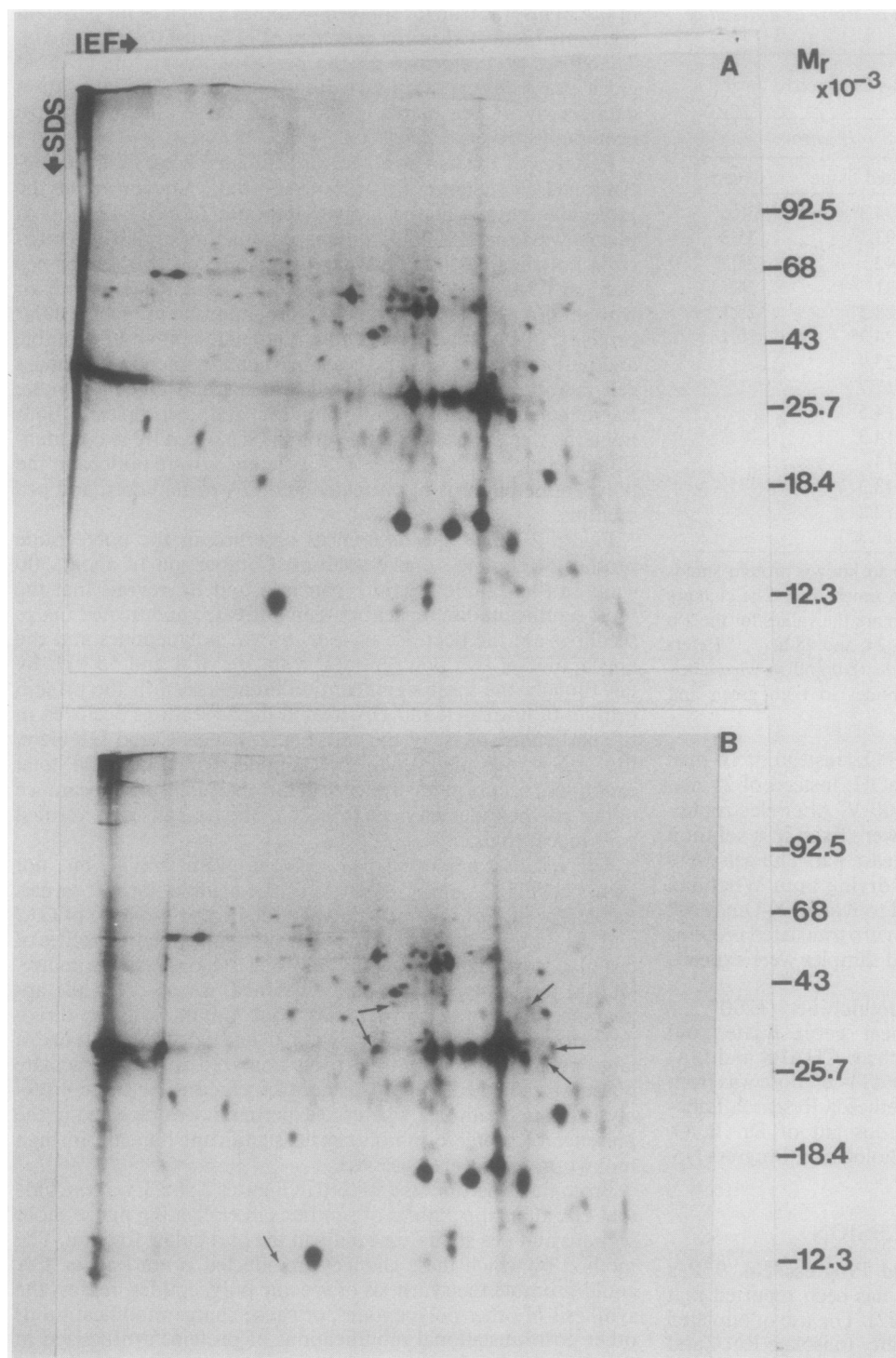
FIG. 5. Fluorographs of *in vitro* translation products poly(A) RNA from light-grown maize seedlings and resolved by two-dimensional gel electrophoresis. Panel (A) shows the translation products from untreated seedlings, (2 h); panel (B) shows the translation products from RNA isolated from seedlings which were treated for 2 h with GA_3. The arrows in panel (B) point to polypeptides whose levels of translatable mRNAs are induced or increased by the presence of GA.

with those observed when GA is applied to the developing aleurone of barley. In the barley aleurone, there is an increase in the activities of several hydrolytic enzymes by 6 to 8 h after treatment with GA_3. Johnson and Kende (11) showed that two enzymes involved in lecithin synthesis in the aleurone of barley are also regulated by GA_3 within 2 h of application of the hormone. It is not known if these two enzymes play a role in GA-mediated stem elongation in corn and peas; however, these two enzyme activities are localized in a crude particulate fraction which was prepared similarly to the ones described here. In each individual instance where the effects of a plant growth regulator has been studied, specific changes in the levels or activities of

only a few proteins are consistently observed. Many of these changes, in turn, are effected at the mRNA level.

Changes in the Populations of Translatable mRNAs in Etiolated and Green Maize Shoot Sections and Green Pea Stems. Although some information about GA-induced polypeptide synthesis can be gained by the *in vivo* labeling experiments described above, such experiments allow one only to analyze the proteins which accumulate or disappear over the labeling period chosen, and are limited by the amount of time required to label the polypeptides sufficiently to do the analysis. Therefore, we purified poly(A) RNA, and translated it *in vitro* in rabbit reticulocyte lysates to assess the polypeptides synthesized at discrete and short

IEF →

SDS ↓

A $M_r \times 10^{-3}$

—92.5

—68

—43

—25.7

—18.4

—12.3

B

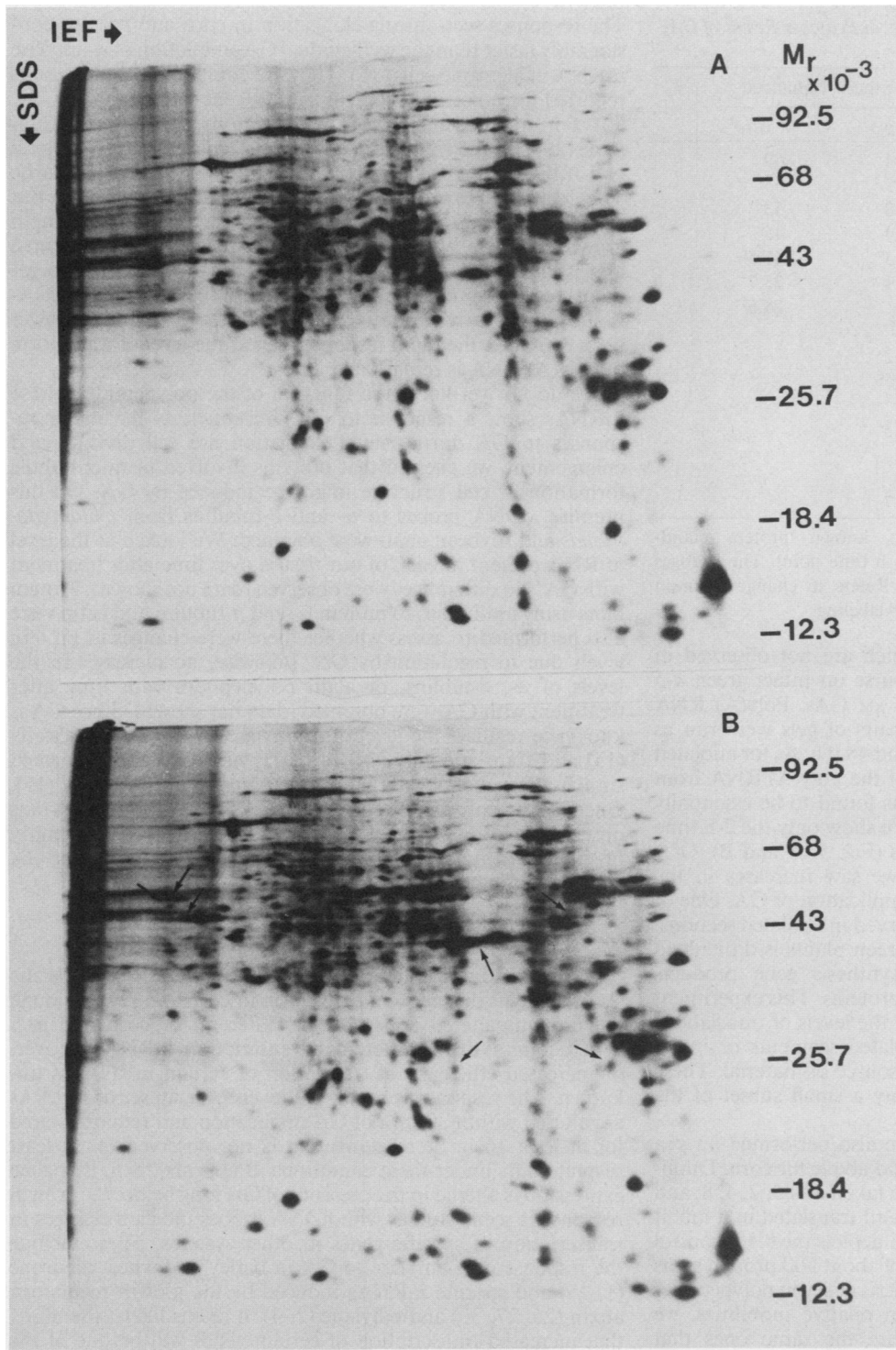—92.5

—68

—43

—25.7

—18.4

—12.3

Fig. 6. Fluorographs of *in vitro* translation products obtained from poly(A) RNA from pea stems and resolved by two-dimensional gel electrophoresis. Panel (A) shows the translation products from untreated stem RNA (30 min); panel (B) shows the translation products from RNA isolated from stems which were treated for 30 min with GA. In panel (B), the arrows point to polypeptides whose levels of translatable mRNAs are induced or increased by the presence of GA.

times after GA application (Fig. 4). Poly(A) RNA was made from experimental (+GA) and control (−GA) tissue treated for 0.5, 2.0, 4.0, 8.0, and 16.0 h. Cell-free translation products of experimental and control RNA for each point were run simultaneously on the same first and second dimension gel apparatus. Duplicate gels for each time point were run. Only protein spots which change in each matched set were scored as being altered by the presence of GA.

The total translational activity of poly(A) mRNA from GA-treated and untreated tissues was found to be essentially the same at all times of sampling (data not shown). For brevity, only those gels from the 0.5 h time point are shown, but the data from all

the time points are summarized in Table I. The autoradiograms from translation products from etiolated *d-5* shoot sections incubated for 0.5 h in the presence of GA₃ indicate that GA caused an increase in 14 polypeptides (Fig. 4B) compared with the control (Fig. 4A). A concomitant decrease in four polypeptides was also observed. None of the changes were dramatic, but the proteins whose levels showed alteration on the 0.5 h gels, continued to show altered levels of expression at subsequent time points. Some of these correlate with polypeptides of similar isoelectric points and M_rs to those proteins whose levels changed during the *in vivo* labeling studies (Table I).

Since the conditions for incubating etiolated shoot sections

Table II. *Summary of Polypeptide Changes in Pea as a Result of GA₃ Treatment*[a]

Synthesis Repressed		Synthesis Enhanced	
In vivo	*In vitro*[b]	*In vivo*	*In vitro*[b]
63.4	None	75.4	60
56.9		74.5	55[c]
56		68.8	53
39.2		57.4	45[c]
39.2		54.3[c]	41.5[c]
32		50.1	25.7
30.6		50	24.6
27.7		44[c]	
24.5		43.2	
23.8		40.5[c]	
23.5		27	
21		23.3	
12.6		23	
10.5		21	
		20.7	

[a] Tabulated are mol wt relative to known protein standards. [b] Shown are the values for the 0.5 h time point. The changes are the same for 2, 4, 8, and 16 h. [c] Refers to changes seen in polypeptides both during *in vivo* and *in vitro* labeling.

may produce artifactual responses which are not observed in intact plants, we repeated the time course on intact green *d-5* seedlings which were sprayed with 10 μM GA₃. Poly(A) RNA was prepared from these plants and pairs of gels were run as described above, at 2.0, 4.0, 8.0, 24.0, and 48.0 h. As for etiolated tissue, the total translational activity of the poly(A) RNA from GA-treated and untreated seedlings was found to be essentially the same at all sampling time points. We show only the 2-h time point, which is indicative of the others (Fig. 5, A and B). Of a total of about 150 spots examined, we saw increases in the synthesis of six polypeptides with the application of GA. One of these spots is in common with one observed in etiolated sections. A comparison between etiolated and green plants is difficult to make since highly abundant photosynthesis gene products masked many of the middle abundant proteins. This experiment indicated that GA causes alterations in the levels of translatable mRNAs in dwarf maize whether etiolated segments or intact light-grown seedlings were used as the source of material. These changes occur quickly and involve only a small subset of the total proteins synthesized.

In vitro translation experiments were also performed on pea Progress No. 9 stem segments as describd above for corn. Duplicate treated and untreated samples were taken at 0.5, 2, 4, 8, and 16 h, and poly(A) RNA was prepared and translated in a rabbit reticulocyte translation system. Figure 6 depicts the 0.5 h control and GA-treated time points. Of a total of about 500 protein spots examined, we saw increases in the synthesis of seven polypeptides with the application of GA. Based on relative mobilities, we believe some of these polypeptides are the same ones that changed during the *in vivo* labeling experiments (Table II). As with the maize *d-5* experiments, these changes are not dramatic, but those changes which appear with 0.5 h exposure to GA remain changed throughout the course of the experiment (Table II).

The results presented in Figures 4 through 6 and Tables 1 and 2 show that GA₃ increases the translational activity in the rabbit reticulocyte system of 6 to 15 poly(A) RNAs within 30 min of application with GA₃. This is true regardless of the experimental system chosen: etiolated corn shoot sections, light-grown corn shoots, or pea stems. A number of mRNAs are rapidly modulated by GA₃ in elongating shoots and stems despite differences in the state of differentiation or development between shoots and stems.

The responses seen during elongation in corn and pea are considerably faster than the well-studied GA₃ induction of α-amylase mRNA in aleurone cells (15). They are comparable to the results reported for auxin-induced changes in gene expression (25, 29, 30). For etiolated maize coleoptile sections, Zurfluh and Guilfoyle (29) showed that auxin altered the translatability of at least two mRNAs by 10 min after application of auxin. Similarly, Theologis and Ray (25) saw an increase in translatability in pea epicotyl segments by 20 min after auxin application. The rapid growth response by plants to auxin has been well-documented and is known to occur within 5 min; growth rate (cell enlargement) reached a maximum within 20 min (25, 27, 30). The GA-induced growth response in plants appears to be much slower (24), therefore, the rapid response seen at the level of translation of poly(A) RNA is remarkable.

We do not yet know the function of the polypeptides whose mRNAs show a response to GA. Inasmuch as the major responses to GA during stem elongation are cell division and enlargement, we guessed that proteins involved in microtubule formation or cell structure might be induced by GA. On this premise, cDNA probes to α- and β-tubulins from *Chlamydomonas* and soybean actin were obtained. We looked at the level of RNA present in each of our tissues over time after treatment with GA. No differences were observed (data not shown). Protein blots using antibodies to human α- and β-tubulin and actin were also performed to assess whether there were changes in protein levels due to regulation by GA. Likewise, no increases in the levels of α-, β-tublins, or actin polypeptides with time after treatment with GA were observed (data not shown). Since GA is known to regulate the level of α-amylase both transcriptionally (15) and translationally (14) in barley aleurone, we performed similar experiments with cDNA to α-amylase from barley (15). Once again, no differences in the level of amylase mRNA was observed due to GA application (data not shown). The identity of the polypeptides which are modulated as a function of GA treatment, therefore, remain unknown.

CONCLUSIONS

Our results indicate that GA alters the synthesis of a specific set of proteins during stem elongation in corn and pea, although the proteins altered appear to be different for corn and pea. Whether this is due to differential transcription, mRNA turnover, or increased efficiency of translation of certain mRNAs is unknown. The response is rapid; the levels of a subset of mRNAs are altered within 30 min of GA application and remain altered for at least 16 h. Stem elongation is not observed for at least several hours under these conditions. It appears, then, that gene expression is altered in the presence of GA long before the growth response is seen. Studies with cDNA probes indicate changes in transcription of specific genes in other systems. These include the response of α-amylase to GA in barley and wheat aleurone (1, 15) and specific mRNAs induced by the growth regulators, auxin (25, 27, 30) and ethylene (2, 4). It seems likely, therefore, that increased transcription of certain genes will be one of the mechanisms involved in GA action during stem elongation. We are currently screening cDNA libraries for genes which are differentially regulated after GA application.

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